Chlamydia trachomatis in women: the more you look, the more you find

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Abstract

***Objective*—To determine the extent to which testing of multiple sites and samples is required to define whether a woman is *Chlamydia trachomatis*-positive.**

***Design*—One-hundred and fifty women attending the Genitourinary Medicine clinic at St Mary's Hospital were enrolled; they had not received anti-chlamydial antibiotics in the previous three months, were not in a high-risk group for HIV infection, or pregnant, or using an intrauterine contraceptive device. Thirty-two women were re-examined three months after recruitment.**

***Methods*—An urethral specimen was Gram stained (smear) and cultured for gonococci. Another urethral specimen was taken to detect *C trachomatis* elementary bodies (EBs) by the MicroTrak direct fluorescent antibody (DFA) test (Syva). An endocervical swab specimen was Gram stained (smear) and cultured for gonococci. One of two other endocervical swabs was used for the DFA test and was then placed in medium which was centrifuged in a MicroCentaur at 13,000 rpm for 10 min; the deposit was examined by using the DFA test. The first 15–20 ml of voided urine (first pass urine; FPU) was also centrifuged and the deposit tested similarly.**

***Results*—Of 182 cervical smears and/or deposits tested for *C trachomatis*, 38 were positive; more cervical deposits (37) than smears (26) were positive and, of these, one-fifth of the deposits and one-third of the smears contained fewer than 10 elementary bodies. Of 162 paired urethral smears and FPU deposits available, one or other specimen of 36 pairs was chlamydia-positive, that is 31 smears and 32 deposits; of these, two-fifths of the smears and half of the deposits contained fewer than 10 EBs. Of 150 sets of cervical and urinary tract samples tested, 31 were chlamydia-positive at both sites, six in the cervix alone and four in the urinary tract alone. Of 139 women for whom there were valid first visit sample results, 36 (26%) were chlamydia-positive in the cervix, 34 (25%) in the urinary tract and 41 (29%) had at least one sample from either site positive. Overall, DFA tests of deposits from centrifuged cervical specimens achieved the highest sensitivity (88%) and those of cervical smears the lowest (70%).**

***Conclusions*—Deposits from centrifuged cervical specimens were *C trachomatis*-positive more often than were cervical smears. Testing deposits from centrifuged urines was as successful as testing urethral smears. One-fifth (cervical deposits) to one-half (urine deposits) of specimens contained fewer than 10 EBs. The urinary tract was chlamydia-positive almost as frequently as the cervix but both sites needed to be tested to define whether a woman was chlamydia-positive.**

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Introduction

Chlamydia trachomatis is the most common aetiological agent of pelvic inflammatory disease (PID), tubal factor infertility and ectopic pregnancy in industrialised societies. Accurate diagnosis and treatment of chlamydial infection in women and their partners is essential if the incidence of these conditions is to be reduced. *C trachomatis* can be detected in approximately 50% of men with non-gonococcal urethritis (NGU)[1] and, in a genitourinary medicine clinic, a similar proportion of women with muco-purulent cervicitis (MPC) was found to be infected.[2,3] While the majority of men with *C trachomatis* infection have symptoms, most infected women are asymptomatic or have minor, self-limited symptoms. Sensitive and specific tests for *C trachomatis* are essential to detect infection in these women.

In our studies, cell culture, a polymerase chain reaction (PCR) and a direct fluorescent antibody (DFA) test have had similar sensitivities and specificities.[4–6] The DFA test can be applied to samples such as urine for which culture methods are unsuitable, and it is our practice to optimise its sensitivity by regarding as positive smears containing one elementary body (EB).

Some investigators have found urine samples to be as reliable as urethral smears for detecting *C trachomatis* in men,[7,8] but the value of examining urines in women is uncertain. We have addressed this issue during a study of the aetiology of MPC, in which detection of *C trachomatis* in urethral smears and urines by DFA staining was compared with its detection in cervical smears and deposits obtained by centrifuging cervical

Table 1 Results of DFA tests on cervical smears in relation to those on deposits from centrifuged cervical specimens

		Number of cervical deposits that were				
		Positive	*Negative*	*Inadequate*	*Unavailable*	*Total*
Number of cervical smears that were	Positive	25	1	0	0	26
	Negative	7	131	2	3	143
	Inadequate	5	5	1	0	11
	Unavailable	0	2	0	0	2
	Total	37	139	3	3	182

samples. In a previous study of women with PID, centrifugation of samples led to an increase in the number of women identified as chlamydia-positive.[9] Thus, by these various means we have attempted to determine the extent to which multiple site and sample testing is required to define whether a woman is chlamydia-positive.

Materials and methods

Subjects

Women attending the Jefferiss Wing (genitourinary medicine clinic) of St Mary's Hospital with a new problem were eligible for enrolment in the study. Subjects were excluded if they had received antibiotics with activity against *C trachomatis* in the preceding three months, if they were in a recognised high-risk group for human immunodeficiency virus infection, if they were pregnant, or if they had an intrauterine contraceptive device. Two investigators (PEH, PJH) enrolled the first three women not fulfilling these exclusion criteria who presented in any single 3-hour clinic session until a total of 150 subjects was reached.

Procedure

Verbal consent to undergo testing was obtained before the women completed a confidential questionnaire regarding symptoms. Samples were obtained as follows. The urethral meatus was cleaned with a saline-soaked gauze and then a 10 μl acetate bacteriology loop was passed 1–2 cm into the urethra. This specimen was used to prepare a smear that was Gram stained and to culture for *Neisseria gonorrhoeae*. A smear for DFA testing for *C trachomatis* was prepared from a fine cotton-tipped swab passed 2-3 cm into the urethra.

A non-lubricated speculum was passed and the cervix cleaned with a cotton-tipped swab. Endocervical material collected on a cotton-tipped swab was used to prepare a smear for Gram staining and to culture for *N. gonorrhoeae*. Specimens for detecting *C trachomatis* were collected by swabbing the endocervix and areas of ectopy on the ectocervix. One swab was expressed in transport medium and stored for future testing, and a second swab was rolled on a slide to produce a smear for the DFA test and then placed in transport medium for the MicroTrak enzyme immunoassay (EIA) (Syva). The order of collecting the two swabs was alternated between clinic sessions. The patient then collected the first 15–20 ml of voided urine (first pass urine; FPU).

Laboratory methods

Handling of specimens Urine samples were stored at 4°C for a maximum of three days. They were warmed to 37°C to dissolve any deposit which had formed on cooling, vortexed to break up threads and to distribute the cell content evenly and centrifuged at 3,000 rpm for 30 min in an MSE Mistral 2000 centrifuge. The deposits were each resuspended in 1 ml of phosphate-buffered saline and stored at −70°C. Prior to testing by the DFA method, these were thawed and centrifuged at 13,000 rpm for 5 min in a microcentrifuge (MSE MicroCentaur). The resulting deposit was resuspended in 20 μl of distilled water and dried on a MicroTrak slide.

One ml of specimen treatment solution was added to each cervical swab to be tested by the MicroTrak EIA (results not presented here). The sample remaining after 200 μl had been removed for the EIA was centrifuged at 13,000 rpm in the MicroCentaur for 10 min. The pellet was resuspended in 20 μl of distilled water and dried on a MicroTrak slide.

Direct fluorescent antibody test The MicroTrak DFA test (Syva, UK) was used as described previously.[4] Urethral smears and urine deposits were fixed in acetone and stained with 15 μl of MicroTrak direct specimen reagent. Specimens were considered inadequate if they contained few epithelial cells in the absence of chlamydial EBs, or if the smear was too thick for individual cells to be brought into focus on microscopy. Smears containing a single EB were recorded as positive.

Table 2 Results of DFA tests on urethral smears in relation to those on deposits from centrifuged first pass urines (FPU)

		Number of FPU deposits that were				
		Positive	*Negative*	*Inadequate*	*Unavailable*	*Total*
Number of urethral smears that were	Positive	27	2	0	2	31
	Negative	5	123	5	13	146
	Inadequate	0	1	0	0	1
	Unavailable	0	1	0	1	2
	Total	32	127	5	16	180

Results

Study population, presentation and diagnosis

One hundred and fifty women were recruited between November 1990 and May 1991. Results of tests for *C trachomatis* were available for samples taken from these women during their first visit and for 32 of them who underwent a follow-up examination three months after recruitment.

The most common reasons for attendance were vaginal discharge (50 women) and having a partner with a *C trachomatis* infection or NGU (29 women). Other common reasons included vaginal soreness (22); dysuria and/or frequency (15); abdominal pain (14); dyspareunia (13); warts (9); genital ulcers (9); gonorrhoea contact (6); asymptomatic, requesting screening (5).

Table 3 Results of DFA tests on urinary tract specimens in relation to those on cervical specimens

		Number of women in whom C trachomatis detection in the cervix was		
		Positive	*Negative*	*Total*
Number of women in whom *C trachomatis* detection in the urinary tract was	Positive	31	4	35
	Negative	6	109	115
	Total	37	113	150

Detection of C trachomatis in the cervix
There were DFA test results for 180 cervical smears and 179 deposits from centrifuged cervical specimens taken from 150 women during their first and follow-up visits. More cervical deposits than smears were recorded as positive (table 1). Thus, seven negative and five inadequate smears had corresponding samples which were positive after centrifugation and for one negative deposit there was a corresponding positive smear. Because of these discrepancies in the detection of *C trachomatis*, a cervix was regarded as chlamydia-negative only if both samples were available and negative. On this basis, 38 cervical samples were considered to be positive (table 1). Eight (32%) of 26 cervical smears and eight (22%) of 37 cervical deposits stained by the DFA technique contained fewer than 10 EBs. Two of these cervical samples contained one and two EBs, respectively.

C trachomatis in the urinary tract
The results of DFA tests for *C trachomatis* were available on 162 paired urine deposits and urethral swabs (table 2). *C trachomatis* was detected in five urine but not urethral samples and in two urethral but not urine samples. In view of this, the urinary tract was regarded as negative for *C trachomatis* only if both samples were available and negative. On this basis, 36 urinary tract samples were considered to be positive (table 2). Seventeen (53%) of 32 urine deposits and 13 (42%) of 31 urethral smears stained by DFA contained fewer than 10 EBs. Four of these urinary tract samples contained one or two EBs.

Concurrence of cervical and urinary tract C trachomatis infection
The frequency with which *C trachomatis* occurred in the cervix and the urinary tract concurrently, or at only one of these sites based on the 150 sets of samples for which DFA results were available is shown in table 3. *C trachomatis* was detected at both sites in 31 (75%) of 41 positive women, in the cervix alone in six (15%) and in the urinary tract alone in four (10%).

Prevalence of C trachomatis in the women studied
Of 139 women for whom valid results were available for samples taken at their first visit, cervical or urinary tract *C trachomatis* infection was detected in 36 (26%) and 34 (25%), respectively. Overall, 41 (29%) had *C trachomatis* detected in at least one sample from either site. Of 130 women for whom results were available for a complete set of both cervical and urinary tract samples, 35 (27%) were positive in the cervix, 33 (25%) in the urinary tract, 29 (22%) at both sites and 39 (30%) in at least one sample from one or other site.

Relative sensitivities of tests on different samples
The relative sensitivities of DFA tests on cervical and FPU deposits and cervical and urethral smears individually, relative to the detection achieved by combining the results for all these samples, are shown in table 4. There were 43 patients for whom at least one sample was positive for *C trachomatis*. One patient had an inadequate cervical deposit, three had inadequate or unavailable urine samples and six had inadequate cervical smears. These samples were, therefore, excluded from the analysis of sensitivity. DFA-staining of centrifuged cervical deposits achieved the highest sensitivity (88%) and such staining of cervical smears the lowest (70%).

Table 4 Sensitivities of DFA tests for C trachomatis on different samples relative to the detection achieved by combining the results for all samples

Sample	*Sensitivity*	*PVN**
Centrifuged cervical swab deposit	37/42 (88%)	134/139 (96%)
Centrifuged FPU deposit	32/40 (80%)	119/127 (94%)
Urethral smear	31/43 (72%)	134/146 (92%)
Cervical smear	26/37 (70%)	132/143 (92%)

*Predictive value of a negative result.

Discussion

The 29% prevalence of *C trachomatis* infection in women attending the genitourinary medicine clinic in our study is considerably higher than the 5–10% usually recorded. Such low figures evidently derive from methods that are less sensitive than the one we have used and, furthermore, are based usually on testing only one specimen.

Our results show that DFA-staining of centrifuged cervical samples was 88% sensitive for detecting *C trachomatis* infection, compared with a value of 70% for cervical smears. This enhancement occurred despite reducing the material available by making a smear from the swab on a MicroTrak slide and storing a volume of the sample before centrifugation. Moreover, fewer centrifuged samples than smears were judged to be inadequate. Several factors may contribute to this phenomenon; first, the small diameter of the slide well makes it difficult to smear the whole swab across it and more material may subsequently be removed from the swab by agitating it in a liquid medium than by rubbing it on a glass surface. However, we think that the most important factor must be the increase in concentration of the material available for examination achieved by the process of centrifugation.

When urinary tract specimens are considered, examining the centrifuged deposit from

a FPU by DFA-staining was more sensitive than examining a urethral swab (80% and 72%, respectively). As in men,[10] time since voiding had no influence on the detection of *C trachomatis* in these samples. The observation in this study that even urinary tract samples, stained by the DFA technique, provided greater sensitivity for detecting *C trachomatis* than a cervical smear highlights the inadequacy of using the latter alone for the routine diagnosis of infection.

Of all the women who were chlamydia-positive at one or both sites, 75% were positive in the cervix and the urinary tract concurrently and 10% of them in the urinary tract alone. This suggests that combining samples from both sites should increase the rate of detection of *C trachomatis* in women. However, improvement in the performance of many diagnostic tests, in particular some EIAs, is essential before it will be possible to detect the small number of EBs, that is fewer than ten in a considerable proportion of samples, that has been noted in this study and previously.[11]

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